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BIOLOGICAL CONTROL OF THIOBACILLUS FERROOXIDANS BY PREDATORY ORGANISMS

for

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BIOLOGICAL CONTROL OF THIOBACILLUS FERROOXIDANS BY PREDATORY ORGANISMS

1. INTRODUCTION

This report is submitted in accordance with the terms of DSS Contract Serial Number OSQ 83-00197

2. OBJECTIVE

This program is directed at the application of biotechnology to the control and prevention of acid drainage from mine tailings. The objective of Phase 1 has been to undertake an in-depth evaluation of the published literature on biological interactions which, in the natural environment, will inhibit the growth of Thiobacillus ferrooxidans and hence the generation of acid mine drainage. The technical feasibility of these potential biological agents will be assessed and those organisms and/or techniques of interest will be recommended for study in Phase 2.

3. LITERATURE SEARCH

A computer-assisted literature has been done and appropriate references have been assembled. The literature search has been conducted in two stages. In the first stage, specific biological agents or methods to control acid mine drainage have been sought. The keywords used and the data bases searched are listed in Appendix I. This provided a very limited number of relevant papers and was indicative of the level of development of the technology. In the second stage of the literature search, we concentrated on examining the characteristics of known predatory or parasitic organisms relative to their potential application in the ecological environment of a tailings pile. The feasibility of using these organisms to control the population of T. ferrooxidans is assessed in later sections. In this group was also included the use of techniques to control acid generation by inhibiting the metabolic processes of one or more bacterial species involved in the overall reaction mechanism by which acid is generated from pyritic minerals.

4. BACKGROUND

4.1 Acid Mine Drainage

Acid mine drainage is considered to originate predominantly from the metabolic activity of the autotrophic, iron-oxidizing bacterium, T. ferrooxidans. The ability of this organism to oxidize ferrous iron and reduced sulphur compounds with the production of acidic ferric sulphate solution has been well documented. This acidic solution initiates the oxidative dissolution of sulphide minerals, especially iron pyrite, in ore residues and tailings. T. ferrooxidans can then maintain the dissolution reaction, and the acidity of the environment, by its continued catalysis of the ferrous/ferric oxidation reaction. The result is a serious problem due to the escape of highly acidic effluents into streams and lakes. Often, toxic metals will be leached from the ores by the acid.

During the past two decades, control technologies have received a great deal of attention. Many control methods are based on chemical treatments to neutralize and render insoluble any potentially toxic elements. The tailings must then be stored in a manner to discourage or prevent the growth of T. ferrooxidans. Physical storage methods to create an anoxic environment may involve vegetation cover, capping or deep-water disposal. These methods, however, are sensitive to environmental factors such as rainfall (leaching), evaporation, erosion, seismic activity, etc. Their action may result in the restoration of ecological conditions favourable to the growth of acid-generating bacteria in the tailings. As long-term disposal methods, these storage procedures have many weaknesses.

In light of these limitations for chemical or physical techniques, biological control methods have many attractive features. The agents are themselves isolated from the natural environment and, therefore, do not introduce a foreign substance. By establishing a controlled biological population within a tailings heap (as the tailings are generated), it is hoped to create a self-generating mechanism to prevent the excessive development of acid-generating bacterial communities.

The control mechanisms will involve either the destruction of T. ferrooxidans cells as they reproduce or the creation of an environment which will not support the growth of this organism even when a suitable substrate is present. These agents or approaches include:

a) Active bacterial predators for T. ferrooxidans such as rotifers, ciliated or flagellated protozoa.
b) Parasitic or predatory bacterial species such as Caulobacter or Bdellovibrio.
c) Production of metabolites (by a microorganism) which are toxic to T. ferrooxidans.
d) Isolation of a bacteriophage specific to T. ferrooxidans.
e) Prevention of the gradual lowering of pH within tailings such that T.ferrooxidans will not be active i.e. it requires a pH of less than 4.0.

In our review, we have attempted to include all possible routes to the biological control of the acid-generation pathway. Some consideration has also been given to the ecological and geological factors which will influence the survival of both the acid-generating and the control organisms.

4.2 The Ecology of a Tailings Pile

In current management practices, the tailings, as generated by the mill, may be either slightly below neutral or alkaline. The exact characteristics will depend on the wastewater treatment technologies in use at a mine. If lime is applied to remove metals and neutralize acids, the alkaline sludge will be discharged usually with the tailings. The constituents of the residual ore particles will have a certain ability to neutralize acid. This will depend on the mineralogical composition of the original ore. The ability to sustain the growth of T. ferrooxidans will depend on the presence of reduced iron and reduced sulphur compounds in the tailings e.g. iron pyrite (FeS_2). This too will be dependent on the chemical composition of the original ore.

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The gradual generation of an acidic environment in a tailings heap will be assisted by the leaching action of rainwater. The level of acidity in the rainfall at different regions of the continent will vary and hence the rate at which the neutralization occurs, may be significantly greater in certain localities.

Biological organisms require moisture for growth and survival. The small particle size of tailings will facilitate the retention of water at the particle surfaces as well as in the extensive interstitial spaces. Microorganisms also require a food supply. In the case of the autotroph T. ferrooxidans, there is usually no lack of nutrients i.e. reduced iron and sulphur minerals, in tailing piles. This bacterium is aerobic and during autotrophic growth obtains carbon for the generation of cellular components from atmospheric carbon dioxide. The necessary oxygen and carbon dioxide will be supplied in part by the amounts of these gases dissolved in the rainwater. During periods of low rainfall, upper zones of the tailings will dry out which will permit air to penetrate to subsurface regions.

It has been proposed that the sequence of reactions leading to the creation of an environment favourable for the growth of acid-generating bacteria, such as T. ferrooxidans, may involve other iron-oxidizing microorganisms. Thus, a succession of bacterial communities, each catalyzing a specific stage of the overall reaction sequence, will lead eventually to the establishment of an acid-generating culture. Singer and Stumm (1970) showed that the rate of generation of acid by the dissolution of iron pyrite in coal is dependent on the rate of ferrous iron oxidation. Stumm and Lee (1961) had previously found that the rate of non-biological oxidation of ferrous iron is directly dependent on the solution pH. At pH 3.5-4.5, however, the rate is independent of solution acidity (Singer and Stumm, 1970). The ferric iron produced by these reactions will oxidize iron pyrite.

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T. ferrooxidans requires a pH of less than 3.5 for good growth and at that value the catalytic effect of the organism on the $Fe^{2+} \rightarrow Fe^{3+}$ reaction is significant (Schnaitman et al., 1969). Walsh and Mitchell (1972a, 1972b) identified an iron-oxidizing bacterium of the genus Metallogenium which, they found, would efficiently oxidize ferrous iron in the pH range 3.5-4.5. They proposed that this biologically mediated oxidation of iron would accelerate the slow, abiotic oxidation process in this pH range. They hypothesized that this would create the acidic conditions favourable for the growth of T. ferrooxidans. In their tests, the presence of Metallogenium in culture medium (pH 4) inoculated with T. ferrooxidans led to an active acid-producing culture (pH 2.6) within 45 days, whereas culture medium inoculated with only T. ferrooxidans remained at pH 4 with no detectable growth of the organism.

Walsh and Mitchell (1972b) concluded that the inhibition of Metallogenium activity should interrupt the succession of iron-oxidizing bacteria and prevent the rapid development of acid-generating communities. Further discussion of their investigations will be presented in a later section.

The development of acid-generating cultures in tailings will also be influenced by the presence of organic residues, toxic metals and other constituents from the ore-processing plant. Although, these constituents may inhibit the growth of T. ferrooxidans, the eventual release/mobilization of these toxic components will usually lead to the restoration of conditions able to support the growth of the organism. A process of natural selection may also lead to the presence of metal-tolerant strains.

4.3. A Strategy for Biological Control

A successful biological control agent or method must be able to maintain an ecological environment within the tailings which will inhibit, over a long period of time, the development of a large population of acid-generating bacteria. Two basic approaches are either

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a) the inoculation of a predator for T. ferrooxidans

or

b) the creation of an environment which will not support the growth of T. ferrooxidans.

In the ideal situation, the former approach will be a form of "bacteriostatic" agent. If the rate of predation is too great, the predator will deplete its environment of bacterial cells, leading to its own demise. For long-term control of the situation, the predator should be able to exist in a steady-state with the T. ferrooxidans. Thus, the rate of predation should not exceed the rate of bacterial reproduction but should be able to maintain the microbial population at a non-critical level so that little or no acid production is observed.

In the latter situation, the control agent must, over a long interval, maintain the tailings environment in a condition which is hostile to T. ferrooxidans. If, for example, an approach is based on maintaining the pH value at 5.0 or higher (by the inhibition of Metallogenium), the treatment must also be able to neutralize any acid produced by the abiotic oxidation of ferrous ions to the ferric form which will subsequently precipitate as ferric hydroxide i.e.

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3(ppt) + 3H^+$$

Although this reaction is much slower than the biologically catalyzed production of acid, it may over a long period of time cause the pH within the tailings to drop to a critical level. The development of T. ferrooxidans communities might occur in localized zones of higher acidity.

The effectiveness of either of these control technologies will be influenced significantly by several factors, including the minerology of the tailings; the microecology and interactions between bacteria and particles; the population dynamics of both the endogenous bacteria within the tailings and the inoculated predatorial organisms; the diffusional characteristics of toxic or neutralizing chemicals or metabolites; the moisture balance; the specificity of an agent towards a target organism etc. All of these aspects have been borne in mind during our appraisal of the literature.

5 LITERATURE REVIEW

Our assessment of the literature is divided into two major sections. In the first section, we will report on those techniques which claim to be control technologies. In the second section, we will review some of the fundamental characteristics of known bacterial predators or parasites.

5.1 Biological Control of Acid-generating Organisms Methods and Agents Reported in the Literature

5.1.1 Caulobacter spp.

Shearer et al. (1968) examined samples of the waters from coal mines showing no apparent acid drainage problem. They tested the effect of these waters, which they considered contained natural inhibitory compounds, on the generation of acid by T. ferrooxidans inoculated into a coal pile. Using an upward-flow, column reactor, they found that the inoculation of some non-acidic mine waters caused a significant reduction in the amount of acid produced. Shearer and Everson (1969) attempted to identify the inhibitor(s) present in these waters. Tests for bacteriophage were negative. Caulobacter were identified to be present and several strains of Caulobacter were assessed for their ability to inhibit the growth of T. ferrooxidans (as indicated by the oxidation of iron in specific culture media). These authors also investigated the effect of different types of reactor systems on the inhibition reaction. The best results appeared to occur with an upward-flowing vertical system.

In later studies, Shearer et al.(1970) isolated Caulobacter spp. from the non-acidic mine waters. A strain was adapted to grow in a synthetic acid mine drainage liquor (pH 3.6) and cultured to yield high cell numbers. These Caulobacter cells were diluted and sprayed on to the surface of a settling pond which had iron/sulphur oxidizing bacteria present. The authors stated that after two months the acidity of the effluent from the pond was lower. Although iron and sulphur-oxidizing bacteria were still present, their numbers

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appeared to be reduced. At no time were accurate viable cell counts undertaken on the iron-oxidizing population and this raises some doubts about the validity of the above observation.

The assumption that the inhibitory species was biological in origin was based on the results of plate studies using solid nutrient medium (Shearer et al., 1968). They noted an apparent improvement in inhibitory activity towards T. ferrooxidans following a series of enrichment cultures. The methods and experimental techniques were poorly documented.

Baker and Wilshire (1971) queried whether the apparent inhibition of the acid generation was in fact due to the activity of a Caulobacter sp. They obtained a sample of the culture from Shearer et al. and carried out their own series of tests. They hypothesized that the acclimation of a Caulobacter sp. to grow actively at pH 3.6 was inconsistent with known data on this organism. They noted that Shearer et al. did not clearly demonstrate that the T. ferrooxidans cultures in the coal columns had been allowed to reach dynamic equilibrium prior to the inoculation of Caulobacter cells. Shearer et al. inoculated the coal columns with Caulobacter cells in the original, peptone-containing culture medium i.e. the cells were not washed. Baker and Wilshire (1971) stated that the sudden addition of medium may have resulted in washout of the acid-generating organisms on the coal substrate. There would also be a loading of organic plus inorganic components in the Caulobacter culture medium. Baker and Wilshire concluded from their tests that although the Caulobacter sp. remained constant in numbers, there was no evidence of a reduction in the numbers of iron-oxidizing bacteria nor an increase in the pH. Thus the Caulobacter sp. did not appear to be parasitic towards the chemoautotrophic bacteria. Their observations could, however, be challenged in that they presented no evidence from defined tests, that the Caulobacter sp. obtained from Shearer et al. was still active against T. ferrooxidans. This omission leaves open the possibility that the inhibitory activity towards T. ferrooxidans was not a stable quality and had been lost during the subculturing and maintenance of the Caulobacter strain.

Shearer et al.(1968), and Shearer and Everson (1969) obtained their best results with flooded upward-flow reactors. They did test a horizontal flooded bed reactor and although inhibition was observed to occur, it appeared to migrate only slowly through different zones along the reactor.

Baker and Wilshire (1971) used a horizontal flooded-bed reactor with recycle. Shearer and Everson (1969) did not use recycling of leachate with any of their reactor systems. It is difficult, therefore, to compare accurately the operating parameters used by the respective research groups. Factors such as the number of Caulobacter cells per mL of inoculum, the composition of the feed water, etc., may have been significantly different in the two sets of tests.

The conclusions of Baker and Wilshire cast some doubt on the ability of Caulobacter spp. to control acid mine drainage. However, the strategy followed by Shearer et al.(1968, 1969) is noteworthy in that they attempted to identify why certain coal mines did not appear to generate acidic effluents. Because of the lack of data, we have assumed that the acid-generation potentials of the coals was the same in both the acid and non acid-generating coal mines.

The biochemistry and microbiology of Caulobacter spp. will be considered briefly in a later section of this review.

5.1.2 Interference in Microbial Successions

Walsh and Mitchell (1972a) isolated an acid-tolerant, iron-oxidizing bacterium from iron-bearing streams. This filamentous organism was identified as a Metallogenium sp. These authors hypothesized that this bacterium, whose iron-oxidizing activity was greatest at pH 3.5-4.5, was responsible for creating an acidic environment favourable for the development of T. ferrooxidans. . Data were published confirming the apparent ecological role for this Metallogenium sp.(Walsh and Mitchell,1972b). They

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reported that the iron-oxidizing activity of the Metallogenium sp. was inhibited by increasing ferrous iron concentrations and subsequently (Walsh and Mitchell, 1975) described a control strategy for inhibition of acid generation in a coal mine based on increasing the ferrous iron concentration in the influent waters. The procedure (using ferrous iron concentrations of 100 ppm) was successful in laboratory tests with columns of coal particles.

Subsequently, Walsh (1980) claimed that using this concept it would be possible to control acid generation by recycling mine water (neutralized to pH 4.5) They contended that the ferrous iron content of the effluent would be sufficient to inhibit the Metallogenium and prevent the T. ferrooxidans succession. It should be noted that this inhibitor will not prevent totally the oxidative weathering of pyrite i.e. chemical mechanisms will still be operative above pH 4.5 but the rates of reaction will be much reduced.

5.1.3 General Overview of Reported Control Technologies

Both of the methods described above are classified as "not proven" since promising data obtained by the original researchers have not been duplicated by other laboratories. In order to expand the knowledge base of these methodologies, we shall review the general characteristics of each type of biological control agent.

6.0 FUNDAMENTAL ASPECTS

In this section we will examine the basic ecology and biology of potential control organisms. This will include predatory organisms such as rotifers and protozoa which were not identified in either of the above control technologies.

Control technologies will use either a predator species to prey on the acid-producing bacteria or the creation of a hostile environment to inhibit a succession of biologically induced chemical reactions. Each of these approaches is complex involving not only the population dynamics of predator-prey relationships or the ecological interactions of bacterial communities present in the environment of tailings particles but also the influence of other variables such as moisture level and temperature.

We shall briefly review each of these topics from the standpoint of control of acid-generating organisms in tailings.

6.1 Predator-Prey Relationships

In this context, we shall consider the development of control technologies for T. ferrooxidans based on the use of rotifers or protozoa as biological agents. In those situations, the predator consumes the prey as a food. For the predator to survive within such an ecology, there must be an adequate supply of the food. Thus, in the case of the ciliated protozoon (biflagellate) isolated by Ehrlich (1963) from acid mine water, the protozoon did not multiply until long after the number of autotrophic bacterial cells had reached a maximum. This may indicate a preference by that predator for dead bacterial cells (Seed, 1971). If this observation is correct, the inability of the predator to consume live bacterial cells would not be consistent with an inhibition of the production of acid.

Starkweather et al.(1979) stated "The conventional view among zooplankton ecologists has been that free living bacteria are seldom the primary constituents in rotifer nutrition while they may serve as supplementary or diversifying food sources in the total diet". Pourriot (1965) is stated by these authors, to have been unsuccessful in maintaining long-term cultures of Brachionus calyciflorus on Bacillus rubens or Aerobacter aerogenes alone. This suggested that the bacteria were of limited nutritional value to the rotifer. However, their own studies (Starkweather et al., 1979) showed that B. calyciflorus could be maintained for over 40 generations on A. aerogenes as food source. These authors noted that the bacterial clearance and ingestion rates were also dependent on the food density.

Curds (1973), using computer models, studied the ecology of sewage bacteria (activated sludge) and carnivorous, ciliated protozoa. These latter protozoa feed on bacteria-consuming ciliates and their existence might prove a threat to any control technology using the application of large amounts of ciliated protozoa to tailings. Thus, all of the bacterial-consuming predators are not only selective as to the bacteria on which they will prey but they are also susceptible to predation themselves by larger ciliates, rotifers etc.

Fenchel (1980b) identified bactivorous ciliates as "opportunists" in that they respond to localized situations involving packets of high concentrations of bacterial cells. Low cell concentrations do not support the growth of these ciliates.

Another factor impinging on the predator-prey balance is the presence of parasites of the predators. It has been shown that bdelloid rotifers are susceptible to fungal parasites (Barron, 1980a; Barron and Tzean, 1981; Barron and Szijarto, 1982).

In any study using a predator of T. ferrooxidans as a control agent, the above factors will come into play. More common factors such as the respective reproduction rates of the predator and prey, the specifity of the predator for a particular prey, the temperature, humidity, and the effect of particulates will also impact on the effectiveness of the method. Many of the data on predator-prey relationships (involving bacteria as prey) have been based on aquatic communities. This is true with respect to the observations on rotifers (Oliver, 1967) and flagellated protozoa (Ehrlich, 1963) in acid mine-waters. Tailings, the environment in which a control technology is intended to be applied, will introduce additional constraints. For example T. ferrooxidans associates intimately with the surfaces of pyritic minerals and it is not known how this will affect predator-prey relationships.

It is difficult to envisage a predator having the ability to eradicate T. ferrooxidans. Numbers may be significantly reduced but the potential for continued acid development by remaining organisms will be ever present. This will also apply where predators are introduced to tailings at pH values above the growth range of T. ferrooxidans. Certainly the process of acid generation may be slowed down but it is difficult to see this extending to prevention of acid formation.

6.2 Microbial Communities

This section covers both the relationships between predatory and parasitic bacterial species, such as Caulobacter spp. and Bdellovibrio spp., with T.ferrooxidans. It will also consider the role of microbial succession in the process leading to acid generation in a tailings pile.

The former group involves many of the same limitations as the larger predator organisms. Generally, one observes predatory species existing in ecological balance with each other. Total eradication of prey species is not normal. Instead one identifies significant and wide fluctuations in the numbers of bacterial prey and their predator. This cyclical pattern,typical of a relationship between predator and prey,can be expected in a tailings environment. Mineral particles will also influence the balance between predator and prey bacteria.

Microbial successions leading to the development of acid generation within a tailings pile are of particular interest in that they may offer a control mechanism operational at higher pH values of 5.0-6.5. Once acid-generation is occurring, biological agents active only in this range will lose much of their value. Harrison (1978) studied an artificial pyritic coal spoil over a period of 1-1/2 years. The coal pile was inoculated with a slurry of acidic soil and drainage water from an old coal strip mine spoil. Unfortunately, the experiment was conducted at a temperature of 27^{o}C and on a relatively small scale. The development of strong T. ferrooxidans growth and a drop in pH to 2.6 occurred in higher regions within 8 weeks. Along the littoral zones (5 cm above the water surrounding the mound), the pH remained at 5.0 with heterotrophic bacteria being the predominant species present. Harrison observed the presence of ciliated and flagellated protozoa in the pool but the numbers and the species decreased as the acidity of the pool increased. Harrison was unable to isolate a Metallogenium sp. using the method of Walsh and Mitchell (1972a).

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The claimed role for Metallogenium spp. has been discussed in greater detail in a previous section. Harrison (1978) suggested that organic acids produced by heterotrophic organisms, might be responsible for the sharp reduction in pH to the range that T. ferrooxidans will grow and dominate. Certain thiobacilli, e.g. T. neopolitanus, will oxidize sulphur compounds at pH values close to neutrality with the subsequent production of sulphuric acid.

6.3 Protozoa

At this time, the most direct evidence for active predation of T. ferrooxidans has involved flagellated and ciliated protozoa. These types of organisms have been identified as reducing significantly the numbers of T. ferrooxidans in both liquid cultures and a heap leaching operation with coal (McCready, 1984).

An unidentified zooflagellate was originally observed in a 20L culture of T. ferrooxidans and was responsible for reducing the bacterial cell count from 10^8/mL to 10^2/mL within an 18 hour period at pH 2.3. The predator died out rapidly and attempts to re-isolate the zooflagellate have been unsuccessful.

Flagellated protozoa have long been known to occur in acid mine waters. Ehrlich (1963) isolated a flagellated protozoa by enrichment culture from a copper mine in the Southwestern United States. The organism was co-cultured with iron-oxidizing bacteria in 9K medium (Silverman and Lundgren, 1959). In subculturing tests, the presence of the flagellate was maintained but there was always a lag phase (2 weeks) before the predator was detectable. The pH at that time was 2.0 and the flagellate appeared to feed on the T. ferrooxidans whose population had peaked after 3-4 days. There was no evidence that the flagellate could eliminate the bacterial population.

Seed (1971) was not optimistic regarding the use of ciliated and flagellated protozoa as biological control agents for T. ferrooxidans. They have, however, many attractive features.

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It is generally believed that protozoa contribute materially to the decline of bacterial numbers as polluted waters are purified by natural systems (Alexander, 1971). They are highly motile and appear to respond rapidly to favourable feed densities.

Taylor and Berger (1976) noted that in a pond ecology, cohabiting ciliated protozoa were selective as to the bacteria upon which they could grow. Thus,although all laboratory strains of bacteria used in the study supported the growth of ciliates, the bacterial strains isolated from the pond appeared to be able to "discourage predation" and would not support the growth of the ciliates. This observation is important for the use of ciliated or flagellated protozoa as biological control agents. Care will be required that ciliates are isolated which are active against the wild strains of acid-generating bacteria. This may complicate the cultivation and production of larger quantities of the protozoan species. For example, will the ability to prey on wild T. ferrooxidans be lost if a bacterial strain mutates during cultivation? Another question might be whether or not a particular ciliate may discriminate in its prey selection between different wild strains of T. ferrooxidans. A ciliate effective in one situation may be ineffective at another location.

This specificity is also important in the natural environment. Mallory et al. (1983), proposed that Tetrahymena thermophilus demonstrated a high specificity for Salmonella typhimurium and Klebsiella pneumoniae. They believed that the protozoa will eliminate the prey even when an alternative prey is present at concentrations above the threshold number for active feeding by the ciliate or when the rate of growth of the prey is less than the rate of predation.

Another attractive feature of ciliated and flagellated protozoa is their tendency to encyst when they encounter an unfavourable environment e.g. lack of food, hostile pH, dessication, anaerobiosis, overcrowding, and excretion products of bacteria and other protozoa (van Wagtendonk, 1955). The cysts are

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more tolerant to toxic chemicals, acids, high temperatures and they can withstand long periods of drought (Alexander, 1961). Dried cysts of some species have been found to remain viable for longer than five years. Other studies have shown cysts to survive heat treatments of 100°C (3h) and 70°C (26h) (van Wagtendonk, 1955). When favourable conditions return, the protozoa will excyst and return to an active form. Thus, a protozoa could, in theory, nearly eliminate its prey, encyst and re-emerge when the prey density has been restored to the threshold level. If such a cycle could be established against T. ferrooxidans, the requirement of repeated applications of the control organism would be significantly reduced. The cysts would also represent an ideal form for storage and subsequent application of a predator to tailings.

6.4 Rotifers

Although Seed (1971) implied that rotifers might be more effective than protozoa as control against T. ferrooxidans, no evidence was presented to support this view.

In a lake/pond ecology, rotifers tended to be present in low numbers below the surface of a body of water but present in high numbers in the sand 3-4 metres from the water's edge. They tend to concentrate in the top 3-4 cm of the sand. Alkaline waters generally contain only a few species although they will be present in large numbers. Acidic waters, on the other hand, support many species of rotifers but the sizes of the populations will be small (Pennak, 1953).

The application of rotifers to the control of acid mine drainage poses several questions. Certain rotifers do appear to ingest T. ferrooxidans (Oliver, 1967), but this occurred in mine water in a ditch which would not simulate closely the microenvironment within tailings. Attractive features of rotifers are their ability to withstand extremes of temperature and even low moisture conditions. In the latter case, some species, of the order Bdelloides are able to endure dessication. In this event, there is a drying and shrinking of the body contents and cuticle without the formation of a true cyst. A few bdelloids do form secreted cysts when subjected to drying. In their case, a gelatinous material is secreted which quickly hardens and becomes resistant.

Dessicated rotifers may be kept dry for months, or in some cases years, provided that oxygen is present. Under normal circumstances, dried rotifers can be rehydrated to normal activity within hours (Pennak, 1953).

The above characteristics suggest that rotifers are suited for application as control agents. They have the potential for long term survival under the widely varying conditions likely to occur in a tailings pile throughout the course of the year. Unfortunately, the major stumbling block will be the predator-prey balance. There is as yet, no evidence to confirm or deny the ability of rotifers to control acid-generating bacteria such as T. ferrooxidans. The unanswered question is not the ability of rotifers to prey on this autotrophic organism, but rather whether or not the autotrophic organism can be reduced or prevented from developing to sufficient numbers to produce acidic effluents.

6.5 Caulobacter spp.

Caulobacter comprise a genus of gram-negative, unicellular stalked bacteria (Henrici and Johnson, 1935). By electron microscopy, it was confirmed that this stalk was an outgrowth of the cell (Poindexter, 1964).

It was observed that in mixed cultures where caulobacters were present, the stalked Caulobacter spp. were commonly attached to the cell walls of other microorganisms (Poindexter, 1964). This suggested that the Caulobacter were preying or were parasitic towards the other bacteria. Poindexter, however, concluded that the attachment to other cells is not a parasitic action on the part of the Caulobacter spp. She observed no adverse effect on the support cell i.e no cell lysis and the Caulobacter spp. did not show any signs of enhanced growth.

The work of Shearer et al. (1968, 1970) has been discussed in a previous section. The claimed control mechanisms or acid-generating organisms would not appear to be attributable to the Caulobacter preying on the Thiobacilli.

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Caulobacter spp. are aerobic heterotrophs noted for their ability to grow in environments very low in organic nutrients. They require phosphate for growth and polysaccharides appear to be involved in the mechanism by which the stalk attaches itself to a surface. If a control mechanism could be explained for Caulobacter, it would appear to be an indirect effect, possibly due to the release of organics to which the iron-oxidizing T. ferrooxidans is very sensitive.

Caulobacter generally grow at the surfaces of water, of low organic content and at pH close to neutrality. In this respect, their presence in neutral coal mine water (Browning, 1970) is to be expected. They are widespread in nature and they exhibit a relatively unusual morphology. They are non-toxic and, although it is difficult to envisage the survival of this genus in low pH tailings, the claims by Shearer et al.(1968, 1970) and Shearer and Everson (1969) should be investigated further. In this respect, Baker and Wilshire (1971) did not deny the growth of Caulobacter spp. at pH 3.2 but found rather that they did not inhibit the acid generation. Baker and Wilshire did not confirm the identity of the plaque or their flat bed reactor and as noted earlier, Shearer et al. introduced their inoculum low down in an upward flow reactor. Therefore, the test conditions were not identical.

Based on the above information, doubt remains as to a role for Caulobacter spp.in acid prevention. We do not believe, however, that it should be totally ignored since a by-product of the Caulobacter culture may be inhibitory to biological acid generation..

6.6 Bdellovibrio spp.

Bdellovibrio spp. are bacteria which parasitize other gram-negative bacterial species. Although we did not identify any literature references relating to the activity of Bdellovibrio spp. against T. ferrooxidans the latter organism is gram negative and hence is a potential prey.

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Bdellovibrio spp. are active in sewers, lakes and sewage treatment systems but of the species described in the literature, none appears promising as a biological control of acid drainage from tailings. Bdellovibrio do not survive in anaerobic environments (Fry and Staples, 1974) and are relatively sensitive to toxic chemicals (Varon and Shilo, 1981). The parasitic activity of this organism appears less effective at temperatures below 26°C and the efficiency of the destruction of prey varies significantly with the respective bacterial strain and its physiological condition. The various strains of Bdellovibrio also showed marked variations in specificity and efficiency. None of these limitations is desirable for a biological control agent.

The activity of Bdellovibrio spp. is also subject to predator-prey equilibria. This is the case with E. coli in wastewaters and it has been concluded that Bdellovibrio has only a minor role in eliminating E. coli from that environment.

At the present time, Bdellovibrio does not appear to be a promising control agent for T. ferrooxidans.

6.7 Bacteriophage

Our literature review included a search for potential phage against T. ferrooxidans. None has been identified in our current search although Shearer and Everson (1969) did investigate the possibility that an inhibitor of iron-oxidizing bacteria was a phage. However, as a control agent, a virus effective against T. ferrooxidans would have tremendous potential.

6.8 Metallogenium spp.

In the above sections, we have considered known predators of bacteria and have reviewed briefly a few specific attributes of each type of organism as a potential control agent.

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Another route to control of acid-generating organisms is the interruption of the pH succession of microbial communities (Walsh & Mitchell, 1972b). Despite the lack of the confirmatory experimental studies needed to verify those claims, the succession appears to centre around the species Metallogenium. We will review briefly its characteristics as they apply to biological control routes.

A Metallogenium sp. has typically an optimum pH for growth at 4.1 but will grow over the range 3.5-5.0 (Walsh and Mitchell, 1972a, 1972b). Metallogenium will oxidize ferrous iron as an energy source. It has a peculiar morphology. It tends to occur as filaments of cells (without conventional cell bodies). The filaments break very readily upon shaking and the individual fragments tend to be very small and are not retained by a 0.45 micron filter.

Metallogenium spp. will grow in the presence of 0.4% formalin whereas T. ferrooxidans will not (Walsh and Mitchell, 1972b). This ability is useful in isolating the organisms by enrichment culture.

Walsh and Mitchell (1972b) identified a role for Metallogenium in a pH dependent succession of iron bacteria loading in a tailings pile to a dominant culture of acid-generating species (T. ferrooxidans). They proposed several routes to biochemical control of acid generation (1972c). In addition to the use of ferrous iron concentrations (e.g. 100 ppm) to inhibit the growth of Metallogenium as discussed earlier, they suggested:

1. Application of surface active agents to dislodge Metallogenium stalks from active sites.
2. Seeding or inducing growth of an organism antagonistic to Metallogenium stalks.
3. The use of a toxic agent specific for Metallogenium.

7. CONCLUSIONS

We have completed an in-depth review of the literature pertaining to the use of biological agents to control acid generation in pyrite-containing tailings. This study has included a critical assessment of not only organisms which may utilize T. ferrooxidans as prey, but also those approaches based on the inhibition of a key reaction in the succession of reactions leading to the oxidation of pyrite minerals in tailings and the release of sulphuric acid.

We have identified several methods to be examined further at the laboratory level. The development of these methods is not far enough advanced to warrant, at this time, an assessment of factors such as the effect of process parameters, economics, environmental safety and related technological parameters.

Based on available data, predatory organisms are ranked ahead of other approaches. In the literature, data on the predatory activity of rotifers and protozoa against T. ferrooxidans are restricted to a few isolated and poorly documented observations. These predators, however, have been identified as either cohabiting and preying on T. ferrooxidans in acid mine water or eliminating T. ferrooxidans from microbial leaching operations.

Rotifers and protozoa have many attractive features including their ability to withstand relatively severe environmental stresses such as dehydration, shortage of nutrients, low or high temperatures, acidity, etc. This may involve either dessication in the case of rotifers or the formation of cysts in the case of protozoa. These organisms may show a high level of specificity towards prey bacteria and can cause significant reductions in bacterial numbers over relatively short time intervals. There are, however, many factors which must be investigated at the bench.

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We also identify several other approaches which warrant further studies. In general, the experimental data for these methods are incomplete and often contradictory. In some situations, such as the application of Caulobacter spp. or the inhibition of Metallogenium, the positive claims of the originators of the work were not duplicated by other laboratories. This appears to have discouraged the undertaking of additional studies. We do not consider that the evidence, as published, for either of these approaches is sufficiently conclusive to accept or reject them. We recommend that they should be investigated further at the laboratory level in order to obtain more definitive data.

These methods (i.e. the use of Caulobacter or inhibition of Metallogenium) would benefit from limited studies at a more basic level. They represent unique strategies for control of acid generation which, although not validated to-date by other laboratories, may lead to practical technologies.

On the basis of current evidence we do not regard Caulobacter spp. as predators or parasites. The data on this organism as published by Shearer et al. (1968, 1969, 1970) are confusing. The claimed inhibition may not have been due to Caulobacter, which would explain the contradictory evidence from Baker and Wilshire (1971).

Similarly, a role has yet to be confirmed for Metallogenium spp. in the succession of iron-oxidizing bacteria leading to acid generation in tailings etc. It is, however, one explanation for the mechanism by which the pH in the tailings can be reduced more rapidly to the value that favours growth of T. ferrooxidans.

The feasibility of using other predatory organisms, such as Bdellovibrio spp. or viruses has not been indicated by any data in the literature.

RECOMMENDATIONS FOR FUTURE STUDY

McCready (1984) has identified a flagellated ciliate in a coal heap-leach operation. This organism is depleting the T. ferrooxidans cells and would be ideal for immediate investigations. The initial laboratory tests would involve studies to evaluate the performance of the ciliate against T. ferrooxidans in column reactors of both tailings and coal.

Studies should also include a comparison of methods to isolate and maintain viable cultures of the ciliate.

As part of a continuing program, we recommend that samples of tailings and acid drainage from the Nordic Tailings (Elliott Lake) should be obtained. They will be examined for the presence and isolation of possible protozoan predators of T.ferrooxidans. Acid mine drainage from other locations should also be investigated.

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APPENDIX I

APPENDIX I

COMPUTER-ASSISTED LITERATURE SEARCH FOR PREDATORS - KEYWORDS

Caulobacter
Bdellovibrio
Rotifer
Review
Prey
Predator
Parasite
Biological Control
Phage
Bacteriophage
Thiobacillus
Iron-oxidizing Bacteria
Leaching
Tailings
Acid
Mine
Inhibit
Virus
Protozoa
Ciliates
Interaction
Metallogenium

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APPENDIX II

APPENDIX II

BIBLIOGRAPHY

Abieva, R.M. (1971).
"On a New Biologic Agent for Natural Self-purification of Water Bodies".
Hyg. Sanit. 36, 87-91.

Afinogenova, A.V. and V.A. Lambina. (1977).
"The Effect of Temperature on the Dynamics of Interaction Between Bdellovibrio Bacteriovorus and the Host Bacterium". Mikrobiologiya. 46, 741-745.

Afinogenova A.V., Z.R. Penabad and V.A. Lambina. (1982).
"Host Range Characterization of the Genus Bdellovibrio". Biologia (Bratisl). 37, 327-334.

Afinogenova, A.V., A.N. Shkidchenko and V.A. Lambina. (1979).
"The Dynamics of Interaction Between Bdellovibrio bacteriovorus and the Host Bacterium Under Aerobic and Anaerobic Condidtions". Mikrobiologia. 48, 102-105.

Alexander, M. (1961)
"Introduction to Soil Microbiology". Wiley and Sons, N.Y

Alexander, M. (1971)
"Microbial Ecology". Wiley and Sons, N.Y.

Arakelyan, R.N. and D.I. Nikitin. (1981).
"Dynamics of Oligotrophic Bacteria Development in Gamma Irradiated Soils".
Pochvovedenie. 1, 169-172. (Abstract Only).

Arumugan, P.T. and J.I. Furtado. (1980).
"Physico-chemistry, Destratification and Nutrient Budget of a Lowland Eutrophicated Malaysian Reservoir and Its Limnological Implications".
Hydrobiologia. 70, 11-24.

ORF

Babich, H. and G. Stotsky. (1982).
"Nickel Toxicity to Microbes: Effect of pH and Implications for Acid Rain". Environ. Res. 29, 335-350.

Baker, R.A. and A.G. Wilshire. (1971).
"Evaluation of Caulobacter as an Inhibitor of Acid Mine Drainage Formation". Proc. 26th Industrial Waste Conf. Purdue Univ. Pt.1, 41-49.

Baker, R.A. and A.G. Wilshire. (1970).
"Microbiological Factor in Acid Mine Drainage Formation: A Pilot-Plant Study". Environ. Sci. Technol. 4, 401-407.

Barron, G.L. (1980a).
"Fungal Parasites of Rotifers Harposporium". Can. J. Bot. 58, 2193-2199.

Barron, G.L. (1980b).
"The Biological Role of Rhopalomyces magnus". Mycologia. 72, 427-430.

Barron, G.L. and S.S. Tzean. (1981).
"A Sub-cuticular Endoparasite Impaling Bdelloid Rotifers Using Three-pronged Spores". Can. J. Bot. 59, 1207-1212.

Barron, G.L. And E. Szijarto. (1982).
"Structure and Biology of a New Hyphomycete Parasitic on Rotifers". Can. J. Bot. 60, 1212-1215.

Bateman, L.E. and C.C. Davis. (1980).
"The Rotifera of Hummock-Hollow Formations in a Poor Mesotrophic Fen in Newfoundland, Canada". Int. Rev. Gesamten. Hydrobiol. 65, 127-153.

Bender, R.A. (1981).
"Improved Generalized Transducing Bacteriophage for Caulobacter crescentus". J. Bacteriol. 148, 734-735.

ORF

Bendis, I. and L. Shapiro. (1970).
"Properties of Caulobacter Ribonucleic Acid Bacteriophage Cb5". J. Virol. 6, 847-854.

Browning, J.E. (1970).
"Freshening Acid Mine-Waters". Chem. Eng. Jan.12. 40-42.

Buchsbaum, R. (1976).
"Lesser Lights - Rotifers". In Animals Without Backbones. Univ. of Chicago Press, Second Edition. pp.168-172.

Buikema, A.L., E.F. Benfield and C.A. Pittinger. (1983).
"Effects of Pollution on Freshwater Invertebrates". J. Water Pollut. Control. Fed. 55, 829-833.

Burczyk, J., I. Zontek and N. Szurman. (1978).
"Partition of Various Algal Strains in Two-polymer Phase System of Dextran and Polyethyelene Glycol". Bull. Acad. Pol. Sci. Ser. Biol. 26, 745-750. (Abstract Only).

Burnham, J.C. (1977).
"Bacterial Control of Aquatic Algal Populations". U.S. NTIS Report No. PB 276 569.

Burnham, J.C. (1981).
"Bacterial Control of Aquatic Algal Populations - Phase II". U.S. NTIS Report No. PB 82-202060.

Cover, W.H. and S.C. Rittenberg. (1982).
"Effect of Bdellovibrio bacteriovorus Attack on the Surface Hydrophobicity of the Substrate Cell". Abtsr. 82nd Ann. Meeting. ASM., Atlanta. Abstract K51. (Abstract Only).

ORF

Curds, C.R. (1973).
"A Theoretical Study of Factors Influencing the Microbial Population Dynamics of the Activated-Sludge Process. I. The Effects of Diurnal Variations of Sewage and Carnivorous Ciliated Protozoa". Water Res. 7, 1269-1284.

Dumont, H.J. (1977).
"Biotic Factors in the Population Dynamics of Rotifers". Ergeb. Limnol. 8, 98-122.

Ehrlich, H.L. (1963).
"Microorganisms in Acid Mine Drainage from a Copper Mine". J. Bacteriol. 86, 350-352.

Ely,B., A.B.C. Amarasinghe and R.A. Bender. (1978).
"Ammonia Assimilation and Glutamate Formation in Caulobacter crescentus". J. Bacteriol. 133, 225-230.

Evans, W.A. (1981).
"Spatial Dispersion, Prey Selection and Fecundity in the Sessile Rotifer Cupelopagis vorax". Am. Zool. 21, 932. (Abstract Only).

Fenchel, T. (1980a).
"Suspension Feeding in Ciliated Protozoa: Functional Response and Particle Size Selection". Microb. Ecol. 6, 1-11.

Fenchel, T. (1980b).
"Suspension Feeding in Ciliated Protozoa: Feeding Rates and Their Ecological Significance". Microb. Ecol. 6, 13-25.

Finance, C., R. Dinev and L. Schwarzbrot. (1982).
"Spontaneous Bacteriolysis in River Water". Biol. Bull. Acad. Sci. USSR. 8, 494-501.

ORF

Friedberg, D. (1977).
"Effect of Light on Bdellovibrio bacteriovorus". J. Bacteriol. 131, 399-404.

Fry, J.C. and D.G. Staples. (1973).
"Factors Which Influence the Enumeration of Bdellovibrio bacteriovorus in Sewage and River Water". J. appl. Bact. 36, 1-11.

Fry, J.C. and D.G. Staples. (1974).
"The Occurrence and Role of Bdellovibrio bacteriovorus in a Polluted River". Water Res. 8, 1029-1035.

Fukusho, K. (1980).
"Mass Production of a Copepod, Tigriopus japonicus, in Combination Culture with a Rotifer Brachionus plicatilis, fed W-Yeast as a Food Source". Bull. Jap. Soc. Scient. Fish. 46, 625-629. (Abstract Only).

Ganf, G.G., C.J. Merrick and R.J. Shiel. (1983).
"Parasitism: The Possible Cause of the Collapse of a Volvox Population in Mount Bold Reservoir, South Australia". Aus. J. Mar. Freshw. Res. 34, 489-494.

Gilbert, J.J. (1977).
"Defenses of Males Against Cannabalism in the Rotifer Asplanchna. Size, Shape and Failure to Elicit Tactile Feeding Responses". Ecology. 58, 1128-1135.

Gilbert, J.J. (1978).
"Selective Feeding and Its Effect of Polymorphism and Sexuality in the Rotifer Asplanchna sieboldi". Freshw. Biol. 8, 43-50.

Gilbert, J.J. (1980a).
"Further Observations on Developmental Polymorphism and Its Evolution in the Rotifer Brachionus calcyflorus". Freshw. Biol. 10, 281-294.

ORF

Gilbert, J.J. (1980b).
"Observations on the Susceptibility of Some Protists and Rotifers to Predation by Asplanchna girodi". Hydrobiologia. 73, 87-91.

Gilbert, J.J. (1980c).
"Feeding in the Rotifer Asplanchna: Behaviour, Cannabalism, Selectivity, Prey Defenses, and Impact on Rotifer Communities". In Evolution and Ecology of Zooplankton Communities. Edited by W.C. Kerfoot. Univ. Press of New England, Hanover. pp.158-172.

Gilbert, J.J. and C.E. Williamson. (1978).
"Predator-Prey Behaviour and Its Effect on Rotifer Survival in Associations of Mesocyclops edax, Asplanchna girodi, Polyarthra vulgaris and Keratella cochlearis". Oecologia (Berl). 37, 13-22.

Ghilyarov, A.M. (1978).
"Observations on Food Composition of Rotifers of the Genus Asplanchna". Zool. Zh. 56, 1874-1876. (Abstract Only).

Grula, E.A., R.H. Weaver and O.F. Edwards. (1954).
"Studies on a Strain of Caulobacter from Water. II. Nutrition, with Implications for Cytology". J. Bacteriol. 68, 201-206.

Habte, M. and M. Alexander. (1978).
"Mechanisms of Persistence of Low Numbers of Bacteria Preyed Upon by Protozoa". Soil Biol. Biochem. 10, 1-6.

Harrison, A.P.Jr. (1978).
"Microbial Succession and Mineral Leaching in an Artificial Coal Spoil". Appl. Environ. Microbiol. 36, 861-869.

Hendricks, C.W. (19740.
"Bdellovibrio bacteriovorus - Escherichia coli Interactions in the Continuous Culture of River Water". Env. Lett. 7, 311-319.

ORF

Henrici, A.T. and D.E. Johnson. (1935).
"Studies of Freshwater Bacteria. II. Stalked Bacteria, a New Order of Schizomycetes". J. Bacteriol. 30, 61-93.

Houwink, A.L. (1951).
"Caulobacter vs Bacillus spec. div.". Nature. 168, 654-655.

Ibragimov, F.Kh. (1978).
"Determining the Index for Bdellovibrio bacterivorus Bacteria in Bodies of Open Water". Lab. Delo. 427-428. (Abstract Only).

Johnson, R.C., N.B. Wood and B. Ely. (1977).
"Isolation and Characterization of Bacteriophages for Caulobacter crescentus". J. Gen. Virol. 37, 323-336.

Kitajima, C., S. Fujita, F. Ohwa, Y. Yone and T. Watanabe. (1979).
"Improvement of Dietary Value for Red sea Bream Larvae of Rotifers Brachionus plicatilis Cultured with Baker's Yeast, Saccharomyces cerevisiae". Bull. Jap. Soc. Scient. Fish. 45, 469-471. (Abstract Only).

Kleinmann, R.L.P., D.A. Crerar and R.R. Pacelli. (1981).
"Biogeochemistry of Acid Mine Drainage and a Method to Control Acid Formation". Min. Eng. 33, 300-305.

Kolkwitz, R. and M. Marsson. (1909).
"Ecology of Animal Saprobia". Int. Rev. des Gesamt. Hydrolobiologie und Hydrogeographie. 2, 126-152.

Korchak, G.I. and T.G. Omel'yanets. (1980).
"Lytic Action of Bdellovibrio bacteriovorus on Bacteria of the Genus Pseudomonas". Mikrobiol. Zh.(Kiev). 42, 761-765. (Abstract Only).

Kramer, T.T., J.M. Westergaard and A.I. Swann. (1977).
"Monitoring of Waters for Bdellovibrio, a Parasite of Gram-negative Bacteria Present in Sewage". U.S. NTIS Report No. PB 281 792.

ORF

Kume, T. (1979).
"Feeding Patterns of Marine Ciliates Fed on the Heterotrophic Bacteria".
Jap. Mer (Tokyo) 17, 109-116. (Abstract Only).

Lackey, J.B. (1939).
"Aquatic Life in Waters Polluted by Acid Mine Waste". Pub. Health Rep. 54, 740-746.

Lambina, V.A., L.A. Ledova and N.S. Situkhina. (1981).
"Participation of Bdellovibrio in the Processes of Sewage Self-purification".
Mikrobiol. 50, 140-146.

Leetham, J.W., J.T. McNary, J.L. Dodd and W.K. Lauenroth. (1982).
"Response of Soil Nematodes, Rotifers and Tardigrades to Three Levels of Season-long Sulfur Dioxide Exposures". Water Air Soil Pollut. 17, 343-356.

Li, J.L. and H.W. Li. (1979).
"Species Specific Factors Affecting Predator-Prey Interactions of the Copepod Acanthocyclops vernalis with Its Natural Prey". Limnology Oceanogr. 24, 613-626.

Liang, L.N., J.L. Sinclair, L.M. Mallory and M. Alexander. (1982).
"Fate in Model Ecosystems of Microbial Species of Potential Use in Genetic Engineering". Appl. Environ. Microbiol. 44, 708-714.

Lynch, M. (1979).
"Predation, Competition and Zooplankton Community Structure: An Experimental Study". Limnol. Oceanogr. 24, 253-272.

Mallory, L.M., C.S. Yuk, L.N. Liangf and M. Alexander. (1983).
"Alternative Prey: A Mechanism for Elimination of Bacterial Species by Protozoa". Appl. Environ. Microbiol. 46, 1073-1079.

ORF

Marchand, A. (1981).
"An Example of Percolation in Biology: The Predation by Bdellovibrio bacteriovorus". C.R. Hebd. Seances Acad. Sci. Ser. III 293, 377-382.

Marchand, A. and O. Gabignon. (1982).
"Theoretical Model of the Predator-Prey Interaction Kinetics Between Bdellovibrio bacteriovorus and Escherichia coli". Ann. Microbiol. 132, 321-336.

McCready, R.G.L. (1984).
Personal Communication.

McMillan, L. and R. Stout. (1977).
"Occurrence of Sphaerotilus, Caulobacter and Gallionella in Raw and Treated Water". Jour. AWWA. 69, 171-173.

Menshutkin, V.V. and Yu.I. Sorokin. (1980).
"A Model Analysis of the Ecosystem of the Rubinsk Reservoir". Hydrobiologia. 70, 7-10.

Miracle, M.R. (1977).
"Epidemiology in Rotifers". Ergeb Limnol. 8, 138-141.

Mitchell, R. (1971).
"Role of Predators in the Reversal of Imbalances Microbial Ecosystems". Nature 230, 257-258.

Miyamoto, S., M. Hanaoka, Y. Okada and K. Oda. (1979).
"Marine Bdellovibrio Distribution and Its Significance in Ecology of Vibrio parahaemolyticus". Abstr. Ann. Meet. Am. Soc. Microbiol. 79, 35. (Abstract Only).

ORF

Moore, J.W. (1978).
"Some Factors Influencing the Density and Birth Rate of Three Subarctic Rotifer Populations". Archiv. Hydrobiol.83(2), 251-271.

Myers, F.J. (1917).
"Rotatona of Los Angeles, California, and Vicinity with Descriptions of a New Species". Proc. U.S. National Museum 52, 473-478.

Myers, F.J. (1935).
"Three New Brackish Water and One New Marine Species of Rotatona". Trans. Amer. Micros. Soc. 54, 428-432.

Myers, F.J. (1936).
"Psammolittoral Rotifers of Lenape and Union Lakes, New Jersey". Amer. Mus. Novitates 830, 1-22.

Nikitin, D.I. and I.A. Pitryuk. (1983).
"Peculiarities of Physiology of Stalked Bacteria of the Genus Caulobacter". Izv.Akad Nauk SSSR Ser Biol. 1, 293-300. (Abstract Only).

Odelson, D.A., M.A. Patterson and R.B. Hespell. (1982).
"Periplasmic Enzymes in Bdellovibrio bacteriovorus and Bdellovibrio stolpii". J. Bacteriol. 151, 756-763.

Oliver, P.R.M. (1967).
"The Bacteriological Leaching of Copper, and Its Recovery, at Britannia Beach Mine". Camborne School of Mines Mag. 67, 10-19.

Olson, G.J., S.C. Turbak and G.A. McFeters. (1979).
"Impact of Western Coal Mining. II. Microbiological Studies". Water Res. 13, 1033-1041.

Pearre, S.Jr. (1981).
"Feeding by Chaetognatha: Energy Balance and Importance of Various Components of the Diet of Sagitta elegans". Mar.Ecol.Prog.Ser 5, 45-54.

Pennak, R. (1953).
"Freshwater Invertebrates of the United States." First Edition. Wiley Interscience.

Poindexter, J.S. (1964).
"Biological Properties and Classification of the Caulobacter Group". Bacteriol. Rev. 28, 231-295.

Poindexter, J.S. (1981).
"The Caulobacters - Ubiquitous Unusual Bacteria". Microbiol. Rev. 45, 123-179.

Poindexter, J.S. (1981).
"Cellular Differentiation in Mutants of Caulobacter crescentus". Abstr.Annu.Meet.Am.Soc.Microbiol. Dallas, Tex. 81, 95. (Abstract Only).

Poindexter, J.S. (1982).
"Novel Peptidoglycans in Caulobacter spp. and Asticacaulis spp.". J.Bacteriol. 150, 332-347.

Pourriot, R. (1965).
"Recherches Sur l'Ecologie des Rotiferes". Vie et Milieu Suppl. 21, 1-224.

Ramirez, C. and M.Alexander. (1980).
"Evidence Suggesting Protozan Predation on Rhizobium Associated with Germinating Seeds and in the Rhizosphere of Beans Phaseolus vulgaris". Appl. Environ. Microbiol. 40, 492-499.

Roper, M.M.and K.C.Marshall. (1977).
"Effects of a Clay Mineral on Microbial Predation and Parasitism of Escherichia coli". Aus. Microb.Ecol. 4, 279-290.

ORF

Schelling, M.E., S.C.Straley and S.F.Conti. (1979).
"Characterization of Attachment to Prey in 2 Strains of Bdellovibrio bacteriovorus". Abstr. Annu. Meet. Am. Soc. Microbiol. 79, 97. (Abstract Only).

Schnaitman, C,A., M.S. Korczynski and D.G. Lundgren. (1969).
"Kinetic Studies of Iron Oxidation by Whole Cells of Ferrobacillus ferrooxidans". J. Bacteriol. 99, 552-557.

Schoenlein, P.V. and Ely,B. (1983).
"Plasmids and Bacteriocins in Caulobacter spp.". J Bacteriol. 153, 1092-1094.

Seed, L.J. (1971).
"The Control of Ferrobacillus-Thiobacillus Bacteria by Means of Predatory Organisms - A Literature Review". Energy, Mines and Resources, Mines Branch, Extractive Metallurgy Division. Divisional Report EM171-21.

Shearer, R.E., W.A.Everson and J.W. Mausteller. (1968).
"Reduction of Acid Production in Coal Mines with Use of Viable Anti-Bacterial Agents". 2nd Symp. Coal Mine Drainage Res.(Preprints) Mellon Inst., Pittsburgh, Penn. pp.98-106.

Shearer, R.E. and W.A. Everson. (1969).
"Effect of Antibacterial Agents on Mine Drainage". U.S. NTIS Report No. PB 191 215.

Shearer, R.E., W.A.Everson , J.W.Mausteller and R.P. Zimmerer. (1970).
"Characteristics of Viable Anti-Bacterial Agents used to Inhibit Acid-Producing Bacteria in Mine Waters". Proc. 3rd Symp. Coal Mine Drainage Res., Mellon Inst., Pittsburgh, Penn. pp. 188-190.

Shilo, M. and M.Varon. (1970).
"Methods for Separation of Bdellovibrio from Mixed Bacterial Populations by Filtration through Millipore Filters or by Gradient Differential Centrifugation". Revue int. Oceanogr. med., 18-19, 145-152.

ORF

Silverman, M.P. and D.G. Lundgren. (1959).
"Studies on the Chemoautotrophic Iron Bacterium Ferrobacillus ferrooxidans I" An Improved Medium and A Harvesting Procedure for Securing High Cell Yields. J. Bacteriol. 77, 642-647.

Singer, P.C. and W. Stumm. (1970).
"Acidic Mine Drainage: The Rate-determining Step". Science 167, 1121-1123.

Slezacek, A. and A. Rosypalova. (1971)
"Study of Growth Qualities of Desulphovibrio desulphuricans". Biologia I 1, 55.

Stanley, P.M., E.J.Ordal and J.T.Staley. (1979).
"High Numbers of Prosthecate Bacteria in Pulp Mill Waste Aeration Lagoons". Appl. Environ. Microbiol. 37, 1007-1011.

Staples, D.G. (1976).
"Distribution of Bdellovibrio bacteriovorus in Sewage Works, River Water and Sediments". Appl. Environ. Microbiol. 31, 469-474

Starkweather, P.L., J.J.Gilbert and T.M.Frost. (1979)
"Bacterial Feeding by the Rotifer Brachionus calyciflorus: Clearance and Ingestion Rates, Behavior and Population Dynamics". Oecologia 44, 26-30.

Stolp, H. (1979).
"Interactions Between Bdellovibrio and its Host Cell". Proc. Royal Soc. London Series B. 204, 211-217.

Stove,J.L. and R.Y.Stanier. (1962).
"Cellular Differentiation in Stalked Bacteria". Nature 196, 1189-1192.

Straley, S.C. and S.F.Conti. (1977).
"Chemotaxis by Bdellovibrio bacteriovorus toward Prey". J. Bacteriol. 132, 628-640.

ORF

Stumm, W. and G.F. Lee. (1961).
"Oxygenation of Ferrous Iron". Ind. Eng. Chem. 53, 143-146.

Syddal, R.J., K.McGrath, J.Byron and C.Stachow. (1981).
"A New Restriction Enzyme Isolated from Caulobacter crescentus". Abstr. Annu. Meet. Am. Soc. Microbiol., Dallas, Tex. 81, 141. (Abstract Only).

Taleva, Y.G., G.I. Sidorenko, G.A. Bagdasaryan and R. Abieva. (1973).
"A Study of Bdellovibrio bacteriovorus as One of the Biological Factors of Self-purification of Water Bodies". Gig. Sanit. 11-14. (Abstract Only).

Taylor, W.D. and J. Berger. (1976).
"Growth Responses of Cohabiting Ciliate Protozoa to Various Prey Bacteria". Can. J. Zool. 54, 1111-1114.

Thomashow, M.F. and S.C. Rittenberg. 1979).
"The Intraperiplasmic Growth Cycle - The Life Style of Bdellovibrio bacteriovorus". In Studies in Microbiology, Vol. 1. Developmental Biology of Prokaryotes. Edited by J.H. Parish. Univ. of California Press: Berkeley Calif. pp.115-138.

Tomov, A., V. Kassovsky, L. Chorbadjiiska, E. Tsvetkova, N. Tsanev and Z. Vencheva. (1982).
"Lytic Activity of Bdellovibrio bacteriovorus Against Bacteria of the Family Legionellaceae". Zentralbl. Bakteriol. Mikrobiol. Hyg. I. Abt. Orig. A Med. Mikrobiol. Infektionskr. Parasitol. 252, 96-100.

Tudor, J.J. (1977).
"Characterization of Bdellocysts of Bdellovibrio sp. tudor". J. Bacteriol. 131, 314-322.

Tudor, J.J. and S.F Conti. (1978).
"Characterization of Germination and Activation of Bdellovibrio bacteriovorus". J. Bacteriol. 133, 130-138.

ORF

Uematsu, T. and K.I. Ohata. (1979).
"Biological and Ecological Studies on Bdellovibrio 4. Reversible Change of Plaque Forming Efficiency in Bdellovibrio by Passage Through Different Hosts". Ann. Phytopathol. Soc. Jap. 45, 147-155. (Abstract Only).

van Wagtendonk, W.S. (1955)
"Encystment and Excystment of Protozoa". In Biochemistry and Physiology of Protozoa. Vol. 2. Edited by S.H. Hunter and A. Luoff. Academic Press N.Y. pp.85-90.

Varon, M. (1979).
"Selection of Predation-resistant Bacteria in Continuous Culture". Nature. 277, 386- 388.

Varon, M. (1981).
"Interaction of Bdellovibrio With Its Prey in Mixed Microbial Populations". Microb. Ecol. 7, 97-106.

Varon, M. and M. Shild. (1981).
"Inhibition of the Predatory Activity of Bdellovibrio by Various Environmental Pollutants". Microb. Ecol. 7, 107-112.

Varon, M. and B.P. Zeigler. (1978).
"Bacterial Predator-Prey Interaction at Low Prey Density". Appl. Environ. Microbiol. 36, 11-17.

Von Stein, R.S., L.E. Barber and H.M. Hassan. (1982).
"Biosynthesis of Oxygen Detoxifying Enzymes in Bdellovibrio stolpii".
J. Bacteriol. 152, 792-796.

Waigh, F.M., R.L. Stone and W.M Engelmann. (1979).
"Inhibition of Pyrite Weathering by Recycling of Selected Effluents". Proc Coal Conf. and Expo V. Underground Mining Symp., Louisville, Kentucky. pp. 139-146.

Wallace, R.L. (1977).
"Substrate Discrimination by Larvae of the Sessile Rotifer Ptygura beauchampi".
Freshw. Biol. 7, 301-310.

ORF

Wallace, R.L. (1977).
"Adaptive Advantages of Substrate Selection by Sessile Rotifers". Ergeb. Limnol. 8, 53-55.

Wallace, R.L. (1980).
"Ecology of Sessile Rotifers". Hydrobiologia. 73, 181-193.

Walsh, F. and R. Mitchell. (1972a).
"An Acid-tolerant Iron-oxidixing Metallogenium". J. Gen. Microbiol. 72, 369-376.

Walsh, F. and R. Mitchell. (1972b).
"A pH-dependent Succession of Iron Bacteria". Environ. Sci. Technol. 6, 809-812.

Walsh, F. and R. Mitchell. (1972c).
"Biological Control of Acid Mine Pollution". J. Water Pollut. Control Fed. 44, 763- 768.

Walsh, F. and R. Mitchell. (1975).
"Mine Drainage Pollution Reduction by Inhibition of Iron Bacteria". Water Res. 9, 525-528.

Walsh, F. (1980).
"Investigation of the Mechanics of Mine Acid Formation in Underground Coal Mine Drainage". U.S. NTIS Report No. PB 179418.

Westergaard, J.M. and T.T.Kramer. (1977).
"The Role of Bdellovibrio in Biologic Purification of Domestic Waste Water". Abstr. Ann. Meet. Am. Soc. Microbiol. 77, 263. (Abstract Only).

Westergaard, J.M. and T.T.Kramer. (1978).
"A Pilot Study on the Lytic Activity of Bdellovibrio in Wastewater". Water Res. 12, 627-630.

Winkler, M.A. and E.J. Cox. (1980).
"Stalked Bacteria in Activated Sludge". Eur. J. Appl. Microbiol. Biotechnol. 9, 235-242.

ORF

Yelton, D.B., V.F. Gerencser and H.G. Voelz. (1978).
"Isolation of Bacteriophages Active on Hyphomicrobium". Abstr. Ann. Meet. Am. Soc. Microbiol. 78, 237. (Abstract Only).

Zdzislaw, K. and J. Rybak. (1979).
"The Feeding of Chaoborus flavicans Meigne (Diptera, Chaoboridae) and its Predation on Lake Zooplankton". Int. Rev. Gesamten Hydrobiol. 64(3), 361-378.

Zullini, A. and C. Ricci. (1980).
"Bdelloid Rotifers and Nematodes in a Small Italian Stream". Freshw. Biol. 10, 67-72.

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